

16. *Four new species of Gregarines from Mountain Cockroaches of the Cape Peninsula.*
By A. D. HARRISON. (With eleven text-figures.)

The Gregarines described in this paper were taken from the following cockroaches indigenous to the mountains of the Cape Peninsula:

Aptera cingulata (Burm.) 'Table Mountain Cockroach', *Temnopteryx phalerata* (Sauss.) and *Melanosilpha capensis* (Stål.).

Method of Study

The alimentary canal was removed from freshly killed insects, slit open and extracted on a slide in 0.75 per cent solution of NaCl, in which all but one of the species remained viable long enough for examination and measuring. Only mature individuals were measured and, in cases where the gregarines were associated in syzygy, only the anterior partner or primate was measured as the protomerite of the posterior partner, or satellite, is flattened.

Permanent whole mounts of the species examined could not be prepared using absolute alcohol, xylol and balsam as the specimens shrank badly during the dehydrating process; glycerine-jelly mounts were fairly satisfactory but not permanent. The best aqueous mounting medium was found to be 'Gum Chloral'. In this medium specimens were completely cleared without shrinkage; in fact, specimens which had already shrunk during staining, resumed the original shape when the slide was gently warmed. This medium sets hard.

Specimens were fixed in either Schaudin's or Bouin's fixative and stained in Heidenhain's iron haematoxylin which did not wash out in the aqueous medium used.

It was found necessary to cut sections of gregarines *in situ* in the gut of the host so that the epimerite and other features could be studied. The mid-gut was removed from a freshly killed host and put immediately into fixative. After fixing it was treated in hydrofluoric acid, to remove sand-grains, and finally embedded in wax using the methyl benzoate-celloidin method. Sections were stained in Delafield's or Heidenhain's iron haematoxylin and counter-stained.

When sections were stained by the Feulgen method the nuclei of the gregarines did not react though those of the host's gut cells reacted strongly and stained a dark purple. This supported the findings of previous workers.

As cysts of three of the species were discovered it was possible to study their dehiscence. Previous workers have been very definite in stating that cysts

do not dehisce under normal atmospheric conditions but only in air saturated with moisture; some even keep them in a drop of water in a sealed cell while others keep them in a moist chamber. Acting on the advice of Sprague, the cysts were kept in sealed petrie dishes containing just enough water to keep the air saturated; the cysts themselves were not in contact with the water. Under these conditions the cysts dehisced satisfactorily. In the case of two of the species they also dehisced in the open air of the laboratory which was fairly humid, these investigations being carried out during winter and spring when rain was frequent.

Classification

M. E. Watson (1916) gives a very full synopsis of the then-known Families and Genera of the Tribe *Cephalina*, Delage (syn. Legion *Septata*, Lankester), of the Sub-order *Eugregarinae*, Léger, based on the classification of Minchin and Poche. In this synopsis she gives all the essential diagnostic points of each genus. This table was used for classifying these gregarines.

All the species fell into the family *Gregarinidae* Labbé, for which Watson gives the following characteristics: associative or solitary, satellite with septum, epimerite symmetrical and simple, cysts with or without spore ducts. Three definitely, and one almost certainly, fell into the genus *Gregarina* Dufour, for which Watson gives the following characteristics: biassociative, epimerites small and globular or cylindrical, spores dolioform to cylindrical, cysts dehisced by spore ducts.

For differentiation of species Watson gives the following characteristics: size, both medium and average; ratio of length of protomerite to total length; ratio of width of protomerite to width of deutomerite; general shape of the body; shape of the protomerite and of the deutomerite; character of the interlocking device between the sporonts in syzygy; size and shape of the nucleus; colour and character of the protoplasm, and the shape of the cysts and their method of dehiscence.

A species can only be fixed by the above characters when a large number of individuals are considered as there is a great deal of variation between individuals. For instance, mature individuals taken from a heavily infected or from a starved host are not only smaller but also narrower than those taken from lightly infected or from well-fed hosts. (See discussion on the dimensions of *Gregarina gibbsi* from *T. phalerata*.) Also, the protoplasm of starved gregarines is far less dense and less granular than that of well-fed ones.

A far more constant feature that does not vary with nutrition, etc., and which can be used for the differentiation of species, is the structure of the nucleus as it appears when stained in Heidenhain's iron haematoxylin or in other haematoxylin strains. As far as is known, this character has not been used before in the case of this genus.

When stained the nucleus appears to be vesicular and contains a number of clearly visible, darkly staining nucleoli or 'karyosomes'. In the gregarines

described here the number of these varies from one to three in one species, to almost twenty in another; however, in any actual species the number was fairly constant or lay between well-defined limits when mature individuals were considered. Moreover, it appears that these nucleoli vary in staining properties in different species; in three of the species they stained very darkly, almost black, but in the fourth, though clearly visible, they were hardly darker than the rest of the nuclear substance, though the same technique was used. These differences in the distribution of nucleoprotein appeared to be very characteristic, and it would seem probable that other variations would be discovered if more species were to be studied in a similar manner.

The different appearances of the nuclei of the four species are illustrated in figure 11 and further discussed in the descriptions. The use of the nuclear appearance was found most useful in relating starved and well-fed individuals of the same species and also in distinguishing between individuals of the two different species which were found in *Melanosilpha capensis*, especially in serial sections.

Watson, in her general description of the *Gregarinidae*, states that, as the gregarines increase in size, the nucleoli increase in number and decrease in size until they are scattered irregularly throughout the nucleus and cannot be counted. In these species, however, it was found that, though the nucleus of a very small cephalont starts with only one nucleolus which divides up as it grows, it ceases to do so at a fairly early age and each species develops its own characteristic number.

Watson goes on to make the suggestion that this supposed breaking-up of the nucleoli into large numbers before gamete formation, hastens this process and reduces the time that cysts take to develop. She also states that the time taken for cysts to develop and dehisce in the Genus *Gregarina* is two days. In the case of one of the present species the cysts took twelve days to develop and this was the species with over fifteen nucleoli, whereas in the species with one to three nucleoli, the cysts took five days to develop. The time taken for cysts to develop was found to be very constant for each species.

Gregarina fastidiosa n. sp.

Host: *Aptera cingulata* (Burm.)

Figs. 1-3, 11a.

Specimens of *Aptera cingulata* were collected on the lower slopes of the mountain at St. James, Cape Peninsula; most of the specimens found were females and only three males were captured during the whole period (March to June 1943). Nymphs of most instars, except the very earliest, were captured and examined.

These cockroaches live under dense bushes, in crevices in rocks or in any sheltered place into which they can crawl. They are usually found singly but occasionally two or three are found together; the males discovered were with

females. Very young nymphs are sometimes found in groups of four or five, but even these usually occur singly.

All the mature females examined were heavily infected to a greater or lesser degree. In all cases there must have been well over a hundred parasites in the gut; one specimen in particular must have had many hundreds in the mesenteron and hepatic caeca. This specimen died in captivity after a few hours and, when examined 24 hours after death, the gregarines were all alive and in a very active condition. Another interesting case was that of a female which was examined after it had been dead for some days at least as the gut and organs were in an advanced state of decay and were quite unrecognizable;

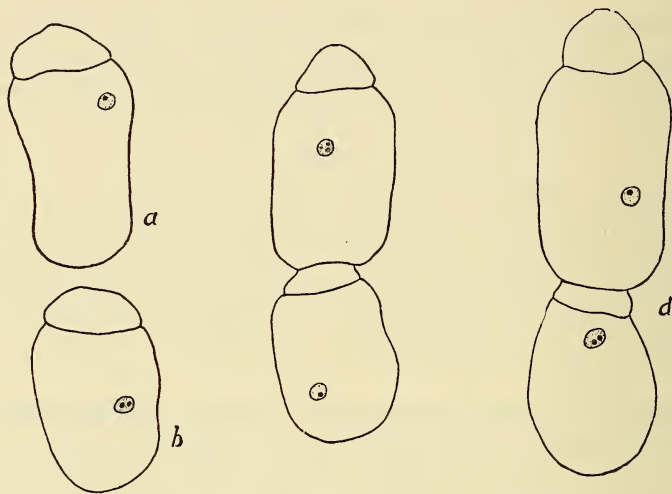


FIG. 1.

Gregarina fastidiosa n. sp., outlines from life, nuclei inserted from sections.
× 46.

here many dead gregarines were found but in moist places there were many still alive.

Cysts were only taken from females and older nymphs.

Of the males examined two were heavily infected but all the parasites were very immature and only cephalonts (i.e. individuals with epimerites) were found.

The guts of the males seemed very empty and it would appear that they do not feed when mature.

The third male examined had very large testes filled with actively motile spermatozoa; the gut in this case seemed to have degenerated and was filled with air bubbles. No parasites were discovered.

All nymphs examined were infected. The earlier instars were lightly infected with young cephalonts while the later instars also contained mature sporonts in syzygy and even had gametocysts in the posterior gut.

Gregarines were found in all parts of the gut except the crop and the gizzard. The hepatic caeca and anterior mesenteron contained mostly cephalonts; the caeca were frequently packed tight with them. Mature sporants, mostly in syzygy, occurred in the middle and posterior mesenteron, a few occurred in the hind gut and some passed out in the faeces.

Cysts were occasionally found in the anterior parts of the mesenteron but there were more in the middle and posterior parts; most cysts, however, were found in the hind gut and in the faeces.

Description

This gregarine is fairly large, quite white in colour, the body is divided into protomerite and deutomerite by means of a well-formed septum and there is a well-marked constriction at the septum. The sporonts are biassociative when in syzygy. The cephalont bears a small, knob-shaped epimerite on a short stalk.

Mature Sporonts (fig. 1a-d). These lie free in the mesenteron, often in syzygy. Dimensions were taken from live specimens but this involved certain difficulties, the chief one being that very few of the specimens on a slide were in a suitable position for measuring as they were either undergoing bending movements, were lumped together, or were adhering to débris. Also specimens did not remain long in the 0.75 per cent NaCl but soon died and became distended; this difficulty was overcome by keeping them in the fluid contents of the gut and by adding as little saline as possible. The forward movement of the gregarines was not very troublesome as this species is very sluggish.

The following figures were obtained from thirty specimens taken from different hosts.

	<i>Average</i>	<i>Medium</i>	<i>Range</i>
Total length	594 microns	582 microns	743-443 microns
Length of protomerite ..	120 "	119 "	143-87 "
Width of protomerite ..	218 "	187 "	229-214 "
Length of deutomerite ..	474 "	463 "	600-400 "
Width of deutomerite ..	352 "	314 "	357-300 "

Ratios required for species determination, based on average figures:

$\frac{\text{Length of Protomerite}}{\text{Total Length}}$	1 : 4.9
$\frac{\text{Width of Protomerite}}{\text{Width of Deutomerite}}$	1 : 1.6

The greatest variation is in the length of the deutomerite (200 microns). The most constant feature is the width of the protomerite—a variation of only 15 microns.

General Shape. The *deutomerite* is more or less oval with the anterior end flattened where it joins on to the protomerite. The greatest width is usually towards the anterior end but not always. The deutomerite often has a very slight waist in the middle and the posterior end is rounded.

The *protomerite* is over twice as broad as it is long.

Cross-sections are quite circular showing that the gregarine is not flattened.

There is a rather unusual variation in the structure of the septum between the protomerite and the deutomerite: thin strands of the septal cytoplasm run forward from the septum into the protomerite and they tend to converge at

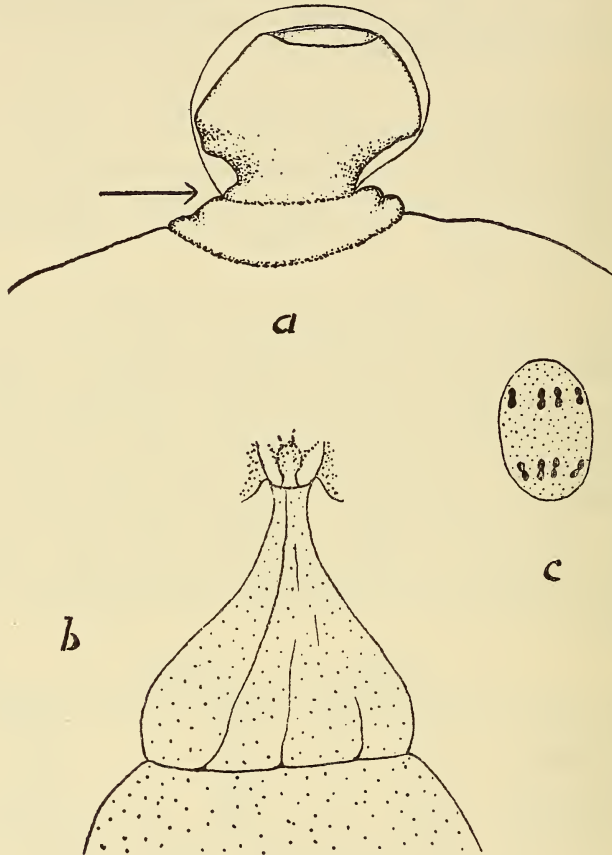


FIG. 2.

Gregarina fastidiosa n. sp. *a.* epimerite surrounded by remains of host cell, arrow indicates point of breakage. $\times 430$. *b.* section showing strands running forward from septum. *c.* stained developing spore from smear, showing eight dumbbell-shaped nuclei. $\times 2000$.

the anterior end. (Fig. 2*b*.) These are only obvious in sections and usually only one appears but occasionally two or three.

The Epicyte. This is rather thick, and thickest in the position of the septum where there is a well-marked constriction. The usual longitudinal ridges appear as well as the myonemes just underneath it.

The Nucleus. This occurs in any position in the deutomerite. It is fairly large and the diameter averages about 60 microns. It contains from one to three nucleoli which stain very darkly in haematoxylin. (Fig. 11a.) Most individuals have two nucleoli; when three are present, one is larger than the other two.

The Interlocking Device. This consists of a raised ridge round the top of the protomerite of the satellite which fits on to the posterior part of the deutomerite of the primite, rather like a sucker. The deutomerite is slightly squeezed out of its normal shape to take it. A characteristic feature of this species is the weakness of this interlocking device; even the gentlest handling separates the sporonts.

The Epimerite. This is knob-like with a short stalk and the entire structure, both knob and stalk, is inside one of the cells of the gut wall. The effect on the host cell is as follows: the cell, normally oblong, becomes round, its striated border disappears and the contents degenerate entirely, neighbouring cells also become shortened, the extent of this depending on their nearness to the epimerite which thus fits into the base of a small pit in the gut wall. (Fig. 3.) In sections the shape of the epimerite is usually obscured by darkly staining degenerated cell contents.

When cephalonts are freed from the gut wall the epimerite is usually left behind but those taken from a host which had been dead for 24 hours came away easily with the epimerite intact surrounded by the gut wall cell which had assumed a spherical shape. The epimerite could be seen inside and appeared to have a slight ridge around the top (fig. 2a); specimens were also found with the epimerite half broken away and the point of breakage is shown in the same figure. The fact that the rounded cell was not the epimerite was not obvious unless specimens were examined very carefully; this could easily lead to erroneous descriptions.

Cysts. These are found in the faeces, hind-gut and a few in the mesenteron. They are oval in shape and shining white in colour; a few spherical cysts were discovered but these did not develop and, when artificially ruptured, were found to contain no spores.

The average size of 20 cysts selected at random from various hosts was 730×438 microns (major and minor diameters). The measurements ranged from 910×450 microns to 500×440 microns. The thinnest cyst was 640×400 microns.

The cysts dehisce by means of from 7 to 12 spore ducts which are from 120 to 140 microns long; the majority of the spore ducts are functional, the spores push through the ducts and form chains often 2 cm. long. In a cyst ruptured artificially after 24 hours, fully formed ducts were formed which stained very darkly in haematoxylin.

It was noticed that when a drop of water collected around a cyst, the cyst burst by means of a simple rupture and released the spores in a cloud which dried into a solid mass.

Cysts dehiscid in five days after extraction from the host. They dehiscid in the sealed petrie dish and seven out of fourteen cysts exposed to the atmosphere also dehiscid. Cysts placed in a sealed petrie dish containing anhydrous calcium chloride failed to dehiscid.

Spores. These are barrel-shaped and very uniform in size, the dimensions being 7.1×4.2 microns. The barrel shape is given by an exterior coat of mucus, and when this was removed in glacial acetic acid, the spores appeared oval. The spore wall could not be penetrated by ordinary fixatives and stains so that sporozoites could not be seen; however, very young spores were obtained by rupturing a cyst artificially after 48 hours; these had no resistant wall and,

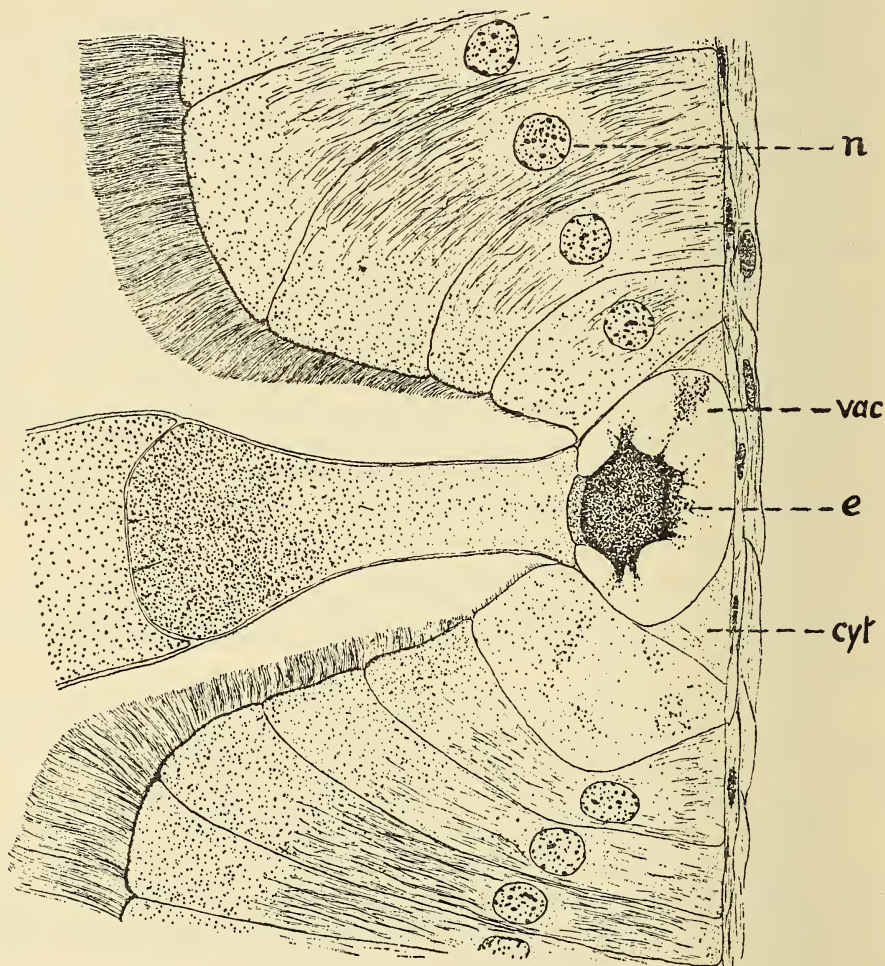


FIG. 3.

Gregarina fastidiosa n. sp., epimerite *in situ* showing effect on host tissues. *cyt.* undegenerated cytoplasm. *e*, epimerite. *n.* nucleus. *vac.* vacuole with degenerated cytoplasm.

when stained, were found to contain eight bilobed nuclei arranged in two rows of four, one row at each pole. (Fig. 2c.)

Gregarina gibbsi n. sp.

Host: *Temnopteryx phalerata* (Sauss.)

Figs. 4-6, 11b.

Specimens of *T. phalerata* were collected on the slopes and summit of St. James mountain during the late autumn and winter. These cockroaches were found under loose stones, pieces of wood and other objects and usually occurred in small groups of three or four and sometimes more.

There appeared to be no striking differences between males and females, as in *A. cingulata*, and, as the degree of infection in both sexes appeared to be much the same they were not dealt with separately as regards statistics.

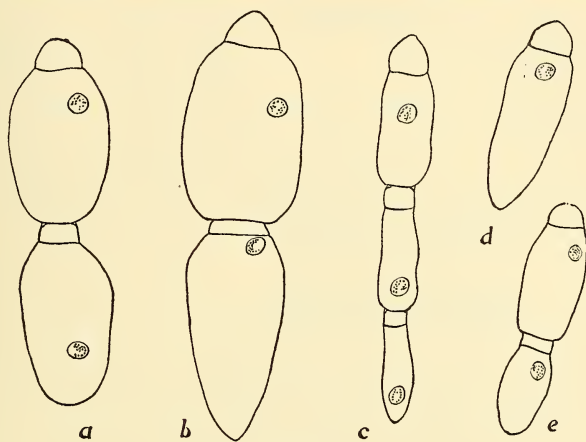


FIG. 4.

Gregarina gibbsi n. sp., outlines from life, nuclei inserted from sections. $\times 46$.

Although these cockroaches were found together in groups, the infection rate was not heavy; 32 per cent of all hosts were infected and only 10 per cent heavily.

The gregarines were found in the anterior mesenteron but none in the hepatic caecae. All cysts were discovered in the hind-gut or rectum.

Description

G. gibbsi is of medium size and is white in colour; it embodies all the general features of the genus. There is a slight constriction at the position of the septum.

Sporonts are biassociative when in syzygy but one unique case was seen where three had associated in line (fig. 4c).

Mature Sporonts. These lie free in the mesenteron and are nearly always in syzygy. Dimensions were taken from live individuals and no difficulty was

experienced in keeping specimens alive in 0.75 per cent saline. Only the primitive was measured.

When this species was first studied and the dimensions and ratios examined, it was found that those from heavily infected hosts were much thinner than those from lightly infected hosts. Before this relationship between proportions and infection rate was noticed it was thought that two species, at least, were involved as the ratios of width of protomerite/width of deutomerite varied from 1 : 1.1 to 1 : 2.6. However, a complete range, linking these two values, was later obtained and the nuclear structure was found to be the same in all individuals. Thus it was concluded that they were all conspecific.

The following figures were obtained from specimens from both heavily and lightly infected hosts.

	<i>Average</i>	<i>Medium</i>	<i>Range</i>
Total length	477 microns	442 microns	614-371 microns
Length of protomerite . .	77 „	71 „	100-57 „
Width of protomerite . .	105 „	86 „	114-86 „
Length of deutomerite . .	400 „	371 „	514-314 „
Width of deutomerite . .	171 „	114 „	227-114 „

Ratios required for species determination, based on average dimensions:

$$\frac{\text{Length of Protomerite}}{\text{Total Length}} \quad \dots \quad 1 : 6$$

$$\frac{\text{Width of Protomerite}}{\text{Width of Deutomerite}} \quad \dots \quad 1 : 1.6$$

The greatest variation is in the length of the deutomerite (200 microns) and the least variation is in the width of the protomerite (28 microns).

General Shape. The shape of the *deutomerite* may vary greatly. In the larger specimens it may be more or less oval and twice or one and one half times as long as broad; in the smaller specimens it may be more elongated and three times as long as broad. The posterior end may be rounded or slightly pointed (fig. 4a-e).

The *protomerite* is dome-shaped and may bear a slight papilla at its anterior end at the position where the epimerite was attached.

The Epicyte. This is moderately thick and tough enough to make handling of live specimens easy. The usual longitudinal ridges are present and myonemes are visible below.

The Nucleus. This occurs anywhere in the deutomerite. The nucleoli stain darkly and are often clumped together so that they are difficult to count. Mature sporonts have from 12 to 20, but the majority have 15. Young cephalonts usually have fewer, 8 or 6 or less (fig. 11b).

The Interlocking Device. This is similar to that described for *G. fastidiosa*. However, here the syzygy is very firm and individuals do not come apart with normal handling and can be easily mounted together.

The *epimerite* is knob-shaped and is borne on a very short stalk. When studied in whole mounts it appears to be slightly flattened at the top around which there appears to be a very distinct ridge (fig. 5). Serial sections showed that in this case not only one host cell was destroyed, as in the previous species, but also several surrounding cells were completely broken down with the apparent disappearance of cell walls (fig. 6).

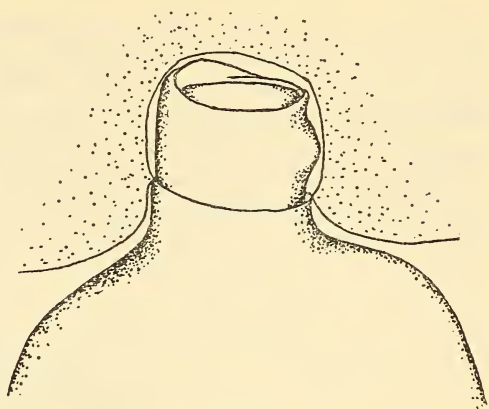


FIG. 5.

Gregarina gibbsi n. sp., epimerite (drawn from whole mount). $\times 360$.

Cysts were found in the hind-gut and faeces. They are oval, white

and glistening. They were not very numerous and few were discovered and only five measured. These gave average dimensions of 428×311 microns and ranged from 457×392 to 400×300 microns.

A characteristic feature is that there is a very thick gelatinous layer outside the true cyst wall; this layer is present in the other species also but is much thinner and hardly noticeable.

The cysts dehiscence in 12 days. Dehiscence took place normally both under normal atmospheric conditions and also in the damp chamber. Six cysts were placed in drops of water in a damp chamber and, after twelve days, only one had dehiscence; the rest were removed and allowed to dry, these dehiscence after two days (fourteen days in all). The inhibiting effect of the water is probably linked with the presence of the thick, gelatinous coat.

Cysts dehiscence by means of from 8 to 12 operational spore ducts which are quite normal in shape. However, when they dehiscence there appear two or three extraordinary ducts of unusual length. These are thinner than usual and appear flattened and no spores are released through them, although the drop of oil that invariably passes up the normal spore ducts just ahead of the spores, also passes up these and hangs on the end. The normal ducts are approximately 250 microns long, while the abnormal ducts are 3,600 microns long.

The spores are exuded in chains, although a few cases were observed where they collected in a large clump at the end of a duct.

The *spores* are barrel-shaped and very uniform in size. They were 8.5×4.2 microns.

Species of *Gregarina* from host: *Melanosilpha capensis* (Stål.)

Specimens of *M. capensis* were collected from the top of St. James mountain during the winter months of 1943. This small, black cockroach was found only

on the top of the mountain in dry situations, usually under loose stones or small rocks perched on top of boulders. They were found usually in small groups of about half a dozen adults, mostly females, with large numbers of small nymphs. The females are apterous but the males, which are few in number, are winged. (Only 7 out of 100 individuals examined were males.)

Two distinct species were found in this host which have been named *Gregarina sandoni* and *Gregarina impetuosa*.

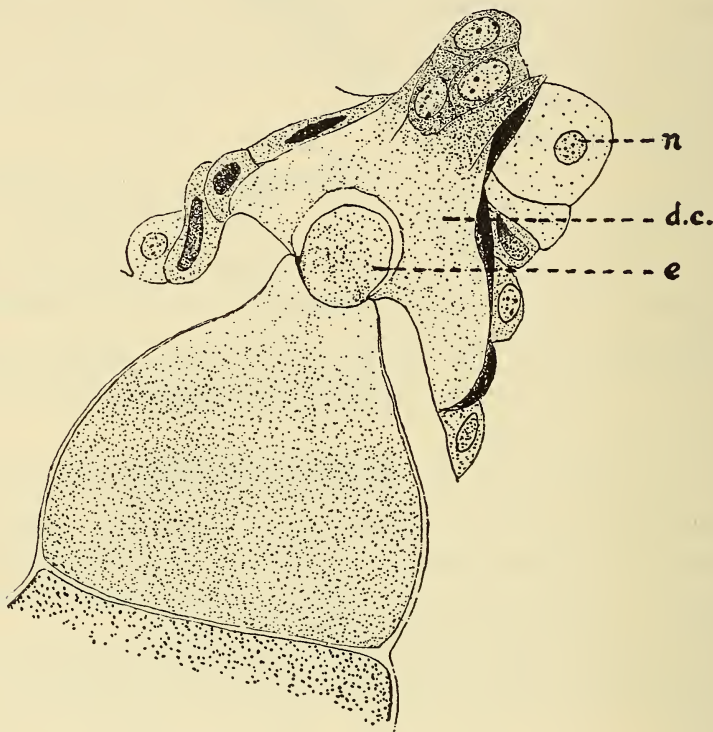


FIG. 6.

Gregarina gibbsi n. sp., section of epimerite *in situ* showing effect on host tissues. *d. c.* degenerated cells. *e.* epimerite. *n.* nucleus. $\times 360$.

Infection. These statistics were obtained from 100 individual hosts:

	<i>Gregarina sandoni</i>	<i>Gregarina impetuosa</i>
Adult of both sexes infected ..	38 per cent	6 per cent
Males infected	29 per cent	14 per cent
Females infected	39 per cent	5 per cent

As will be seen *G. sandoni* was found more often than *G. impetuosa*. Only one host was at all heavily infected with *G. impetuosa* and that was a male. Only 13 per cent of the hosts infected with *G. sandoni* were heavily infected.

Gregarina sandoni sp. nov.

Figs. 7, 11c.

This was found in the anterior and middle parts of the mesenteron and very young cephalonts in the hepatic caeca.

It is a fairly large gregarine, quite white in colour, with a clearly marked septum; there is no marked constriction of the epicyte at the septum but merely a slight dent. The species is extremely sluggish and hardly moves at all.

The sporonts are biassociative, and very characteristic of this species is the very early age at which they form syzygies: young sporonts, only half or even less than half the size of mature sporonts, are commonly to be found in syzygy. These are liable to be mistaken for specimens of *G. impetuosa* but can easily be distinguished by their sluggishness, the structure of their nuclei and the presence of extra-nuclear bodies (which will be discussed later).

Mature Sporonts (fig. 7a-c) were found in the anterior or middle parts of the mesenteron. Dimensions were taken from live specimens and no difficulty was experienced in keeping them alive in 0.75 per cent saline. Only primitives were measured.

The following were the dimensions:

	Average	Medium	Range
Total length	498 microns	451 microns	642-357 microns
Length of protomerite . .	80 „	86 „	100-57 „
Width of protomerite . .	134 „	114 „	157-114 „
Length of deutomerite . .	418 „	443 „	542-300 „
Width of deutomerite . .	274 „	257 „	357-214 „

Ratios for species determination based on average figures:

$\frac{\text{Length of Protomerite}}{\text{Total Length}}$ 1 : 6.2
$\frac{\text{Width of Protomerite}}{\text{Width of Deutomerite}}$ 1 : 2

The greatest variation is in the length of the deutomerite (242 microns) and the most constant feature is the width of the protomerite (43 microns variation).

General Shape. The *deutomerite* is more or less oval in shape but it is slightly pointed at the posterior end. The greatest width is usually towards the posterior end in adults but in younger individuals the anterior is usually the widest part.

The *protomerite* is usually two-thirds as long as it is wide.

The Epicyte. This is very thin and mature sporonts are very easily ruptured even when handled very gently. This feature is not so noticeable in immature specimens. The usual longitudinal ridges and circular myonemes are present. *The nucleus* occurs anywhere in the deutomerite. It is fairly large and averages about 50 microns in diameter. It contains 4 or 5 nucleoli which are rather characteristic as they stain only slightly darker than the rest of the nucleus in

Heidenhain's iron haematoxylin and are often difficult to see. Nevertheless, when viewed in section under an oil immersion lens, they appear as very definite structures with definite outlines. They are often clumped together at one side of the nucleus. (Fig. 111c.)

Apart from the nucleus there is, in the deutomerite, a most characteristic *extra-nuclear body*. This is usually about a quarter the size of the nucleus and is spherical with a very smooth surface. Occasionally it is broken into two, three

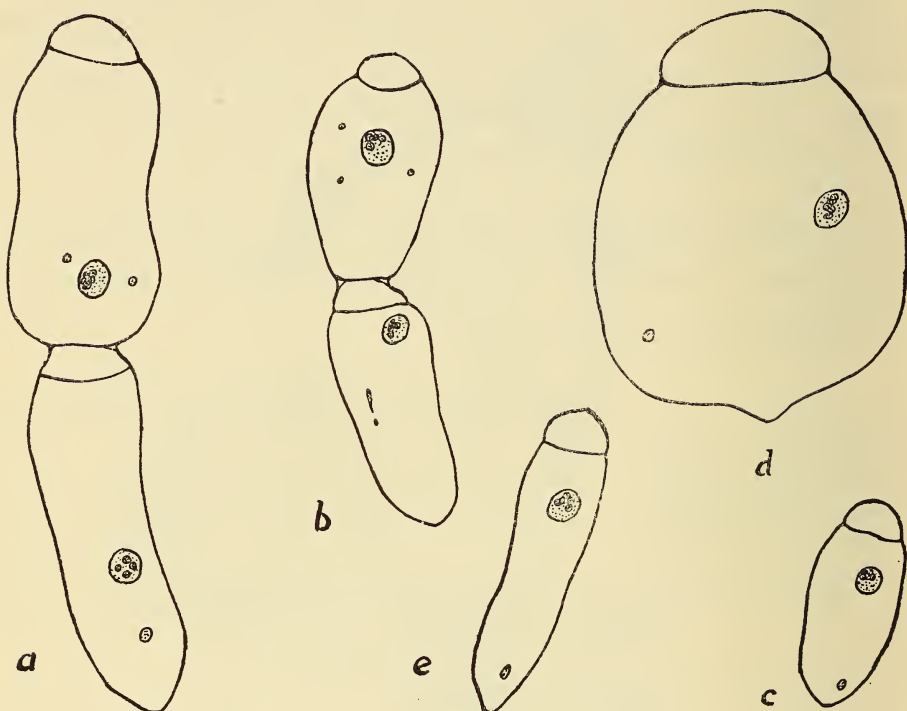


FIG. 7.

Gregarina sandoni n. sp., outlines from life. *a-c.* from normal host. *e.* from starved host. *d.* the only individual in a well-fed host. $\times 76$.

or more smaller bodies and may be elongated instead of spherical. The body stains fairly darkly in haematoxylin but not at all by the Feulgen method. No similar structure was found in the other species nor was any reference found to it, or any similar structure, in the literature. (Typical examples have been inserted in the outline drawings, fig. 7*a-e.*)

The *interlocking device* is similar to that described for *G. fastidiosa*. In this species the syzygy is fairly firm and the primite and satellite are not easily separated with normal handling.

The *epimerite* is of the normal type found in this genus. The whole structure is inside a cell of the gut-wall against which the protomerite is applied. The con-

tents of the cell, including the nucleus, break down and the effect on the surrounding cells is the same as that described for *G. fastidiosa*.

Few specimens were obtained in whole mounts with the epimerite still intact; in fact, this structure seems to be exceptionally easily shed and this may explain why so many immature sporonts are found free in the gut with the early formation of syzygy.

Cysts are oval and are white or light buff in colour. They are found in the faeces and hind-gut. The average dimensions for the shortest and longest diameters are 330 and 500 microns respectively. The cysts range from 282×420 to 420×670 microns. The largest cyst recorded here was very much bigger than the average size but most of the cysts did not vary very much from this average.

Cysts dehiscid in three days after they were removed from the gut. They did not dehiscid when exposed to the atmosphere but only in the damp chamber. Dehiscence was by 7 or 8 spore ducts most of which seemed to be operational. However, when a cyst was artificially ruptured just prior to dehiscence it was found to contain 20 ducts. It would seem that all ducts are not extruded simultaneously and, after the first 7 or 8 ducts are extruded the pressure inside is too low to force the others out.

Spores are dolioform and are extruded in long chains. They are very regular in size and are 7.1 microns long and 3.55 microns wide (measured under an oil immersion lens), exactly twice as long as broad. No success was obtained in attempts to make them exsporulate by putting them in fluid from the mesenteron and hepatic caeca.

An interesting example of the dependence of the gregarines upon the nutrition of their host was discovered when a specimen of *M. capensis* was starved for a month and then opened. The mature sporonts were well below average in size and much thinner, especially the deutomerites. Their average dimensions were:

Total length	464 microns
Length of protomerite			..	64 „
Width of protomerite	104 „
Length of deutomerite	400 „
Width of deutomerite	133 „

One of these starved individuals is illustrated in figure 7e. The endoplasm of these starved gregarines was less dense than normal. Cysts of normal size and shape were discovered but their contents appeared to be coagulated into dense lumps leaving the rest of the cyst transparent. These cysts did not dehiscid and no spores were formed in them.

Figure 7d was the only parasite in an apparently well-fed host and provides an interesting contrast to 7e.

Gregarina impetuosa n. sp.

Figs. 8-10, 11d.

All specimens of this species were found in the anterior mesenteron of the host.

It is a small gregarine with a well-marked septum, at the position of which there is a slight constriction of the epicyte.

The mature sporonts are bi-associative and the cephalonts bear a knob-shaped epimerite on a short stalk.

Most characteristic of this species is its activity; the sporonts glide forwards across a slide covered in gut debris at about 500 microns per minute, pushing the débris aside as they move. This activity made measuring and drawing difficult but it was possible on rare occasions when the gregarines were slowed down by some exceptionally dense obstruction.

Mature Sporonts (fig. 8). These lie free in the mesenteron and are usually associated in pairs. They are unaffected by the 0.75 per cent saline and remain alive in it for long periods.

The following dimensions were taken from the primitive only:

	Average	Medium	Range
Total length	291 microns	300 microns	357-228 microns
Length of protomerite	57 "	57 "	72-43 "
Width of protomerite	66 "	71 "	86-43 "
Length of deutomerite	234 "	243 "	286-185 "
Width of deutomerite	126 "	114 "	171-71 "

Ratios based on averages:

$\frac{\text{Length of Protomerite}}{\text{Total Length}}$	1 : 5
$\frac{\text{Width of Protomerite}}{\text{Width of Deutomerite}}$	1 : 1.9

The feature that shows the least variation is the length of the protomerite (28.7 microns) while the greatest variation is in the length of the deutomerite (101 microns).

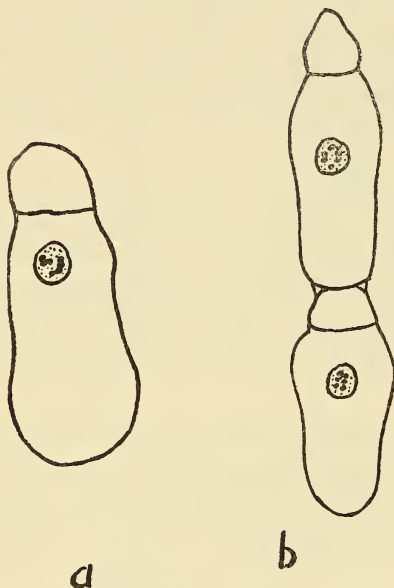


FIG. 8.

Gregarina impetuosa n. sp., outlines from life, nuclei inserted from sections. $\times 100$.

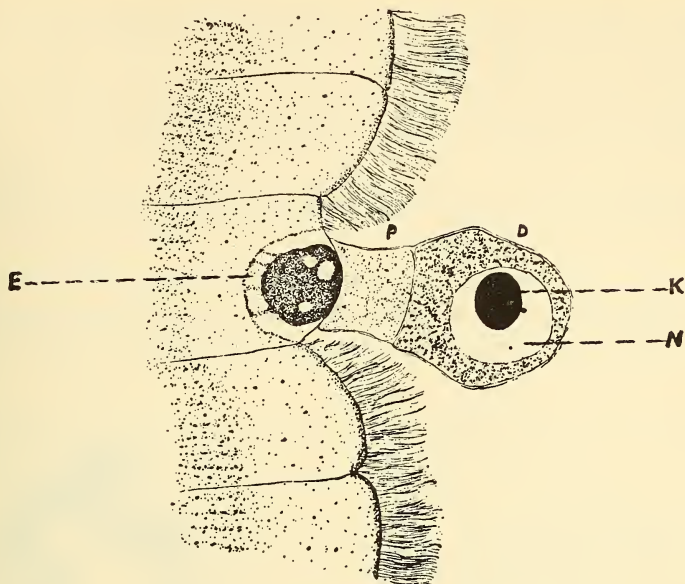


FIG. 9.

Gregarina impetuosa n. sp., showing early development of karyosomes and effect on parasitized cells. This shows the youngest parasite discovered. d. deutomerite. e. epimerite. k. karyosomes or nucleoli. n. nucleus. p. protomerite. $\times 800$.

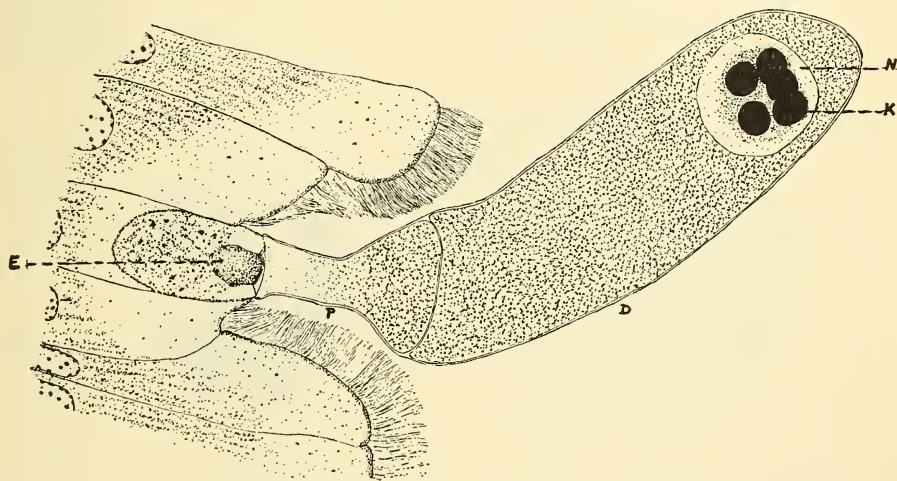


FIG. 10.

Gregarina impetuosa n. sp., a later stage. Lettering as in fig. 9. $\times 570$.